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A Candidate Gene Study of Canine Joint Diseases

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Abstract

Canine osteoarthritis (OA) commonly occurs in association with articular diseases, such as hip dysplasia (HD), elbow dysplasia (ED), or cranial cruciate ligament rupture (CCLR). We hypothesized that a common genomic risk for the development of canine joint disease and canine OA would be identified by evaluating the allele frequencies of candidate gene single nucleotide polymorphisms (SNPs) in dogs with OA associated with different articular diseases when compared with a general population of breed-matched dogs. DNA was extracted from blood samples obtained from Labrador Retrievers and Golden Retrievers surgically treated for ED, HD, and CCLR and confirmed to have radiographic evidence of OA. One hundred and thirteen SNPs in 20 candidate genes were genotyped. No significant associations were identified for SNPs or haplotypes in the candidate genes for the diseases evaluated. The candidate gene approach for the study of genetic association is unlikely to be successful for complex canine diseases such as OA without prior trait mapping evaluation.

Key words: association, canine, gene, joint, osteoarthritis

Osteoarthritis (OA) is a common, debilitating condition of mammalian joints, characterized by the destruction of articular cartilage, resulting in pain and dysfunction of the affected joint. OA is estimated to affect up to 20% of dogs over one year of age (Paradis et al. 2003) in the general dog population. The joints most commonly affected by OA in the dog are the hip, the elbow, and the stifle. Historically, OA of these joints has been considered to be secondary to primary diseases, such as hip dysplasia (HD), elbow dysplasia (ED), and cranial cruciate ligament rupture (CCLR).

Breed risks for the development of HD, ED, and CCLR are marked. The Labrador Retriever demonstrates a 3.4-fold increase in risk for the development of HD (LaFond et al. 2002), a 20.5-fold increase in risk for developing the primary component of ED (LaFond et al. 2002; fragmented coronoid process [FCP]), and a 5.5-fold increase risk for developing CCLR (Duval et al. 1999). Sex predisposition to the development of each of these diseases also exist, with male

dogs being a greater risk for developing hip OA (Hays et al. 2007) and ED FCP; Salg et al. 2006, and neuter status conferring an increased risk for developing CCLR (Whitehair et al. 1993; Duval et al. 1999). The estimates of heritability for HD vary between 0.18 and 0.74 (Reed et al. 2000; Wood et al. 2000, 2002; Todhunter et al. 2003; Janutta et al. 2005), estimates for ED vary between 0.10 and 0.77 (Guthrie 1989; Guthrie and Pidduck 1990; Grondalen and Lingaas 1991; Studdert et al. 1991; Maki et al. 2000), and estimates for CCLR vary between 0.27 and 0.31 (Nielen et al. 2003; Wilke et al. 2006). A genetic correlation between HD and ED has also been identified in a population of Rottweilers (Maki et al. 2000), suggesting that these traits may be influenced by the same genetic and/or environmental factors in certain breeds. As both ED (Lang et al. 1995) and HD (Wood et al. 2002) are phenotyped by radiographic measures including osteophytosis, the genetic risk factors which are common between these 2 diseases may code for a risk of developing osteophytosis.

Recent evidence suggests that genetic factors may additionally affect the development of OA in dogs affected by articular disease. Differences in the breed tolerance of passive hip laxity for the development of hip OA imply that genetic differences affect the development of canine hip OA (Smith et al. 2001). The severity of OA in dogs presenting with ED, HD, and CCLR can vary widely, which is a function of disease duration, animal activity, nutrition status, and genetic profile. Thus, although the significance of primary versus secondary canine OA is undetermined, canine OA per se is likely to have a significant genetic background.

In contrast to human OA (Clements et al. 2006), the genomic basis of joint disease in dogs has received limited investigation to date. A previous case-control candidate gene study of single nucleotide polymorphisms (SNPs) in 4 candidate genes (Fibronectin 1, type 9 collagen alpha 1 chain, type 9 collagen alpha 2 chain, and cartilage oligomeric protein) failed to identify any significant associations between SNPs and the development of CCLR in a population of Newfoundland dogs (Wilke et al. 2005). A study of microsatellite markers adjacent to 14 candidate collagen genes for ED also failed to identify significant associations with the development of the disease (Salg et al. 2006). A microsatellite marker (FH2320) on canine chromosome 3 (CFA3) has been linked with the development of osteophytosis of the cranial and caudal acetabulum in Portuguese water dogs (Chase et al. 2005). In a separate study of a Labrador Retriever and Greyhound cross pedigree, putative quantitative trait loci contributing to macroscopic evidence of hip OA were identified on CFA05, 18, 23, and 31 (Mateescu et al. 2008). Further quantitative assessment of the same pedigree also revealed that hip OA was inherited additively and without dominance (Hays et al. 2007).

The majority of candidate genes studies in human OA have evaluated genes that were associated with the molecular pathogenesis of the disease, such as cytokines and structural components of the extracellular matrix (Clements et al. 2006). The most successful study investigating candidate gene associations with human OA focused on genes that are differentially expressed in human OA synovium and cartilage (Valdes et al. 2004). Subsequently, a number of these gene polymorphism associations have been reproduced in different disease cohorts (Valdes et al. 2007). Furthermore, a prediction of OA risk in individuals could be attained by combining several of the genes that were consistently shown to be involved in OA susceptibility (Valdes, Doherty, et al. 2008).

We hypothesized that genomic risk for the development of joint disease per se and canine OA would be similar across the 3 most common articular diseases affecting dogs (ED, HD, and CCLR) and between different breeds of dog for the same disease (CCLR). We further hypothesized that these genomic risks could be elucidated by evaluating the allele frequencies of SNPs in candidate genes in populations of dogs with ED, HD, and CCLR of a single breed and between 2 breeds with a common disease (CCLR).

Materials and Methods

Candidate Gene Selection

Candidate genes were selected on the basis of previous association of polymorphisms reported with OA in man or from differential gene expression in articular tissues from canine or human OA joints. A full list of the genes evaluated the SNP positions, gene function, and justification for inclusion as a candidate gene are reported in Table 1.

The genes selected were ankyrin repeat domain 10 (ANKRD10), adenosine triphosphatase, Class VI, Type 11B (*ATP11B*), interleukin 1 alpha (*IL1 α*), -2 (*IL2*), -4 (*IL4*), -6 (*IL6*), -10 (*IL10*), -12 β (*IL12 β*), leptin receptor (*LEPR*), matrix metalloproteinase 3 (*MMP3*), -9 (*MMP9*), -13 (*MMP13*), secreted protein, acidic, cysteine rich (*osteonectin/SPARC*), tissue inhibitor of metalloproteinase 1 (*TIMP1*), -2 (*TIMP2*), -3 (*TIMP3*), -4 (*TIMP4*), tenascin C (*TNC*), tumor necrosis factor α (*TNF α*), Zinc finger, and SWIM-type containing 2 (*ZSWIM2*).

Cohort Collection

Genomic DNA was extracted from residual clotted and ethylenediaminetetraacetic acid (EDTA) preserved blood samples using a standard phenol-chloroform extraction method. Samples were suspended in Tris-EDTA and normalized to 5 ng/ μ l. All case samples were obtained from the United Kingdom DNA Archive for Companion Animals (http://pcwww.liv.ac.uk/DNA_Archive_for_Companion_Animals), and all control samples were obtained from a population of breed-matched dogs from the United Kingdom undergoing vaccination. Control dogs were not phenotyped for disease. The breeds and orthopedic diseases evaluated were selected by choosing cohorts for which at least 30 samples had been collected from individuals within a single breed, for a given condition. All samples from cases were collected by veterinary orthopedic specialist surgeons from dogs surgically treated for ED, HD, or CCLR and with no clinical evidence of a concurrent orthopedic condition (ED, HD, or CCLR) at the time of treatment. All cases had radiographic evidence of OA of the affected joint at the time of surgery. Samples were collected from Labrador Retrievers and Golden Retrievers being surgically treated for ED (FCP; Labrador Retrievers, $n = 81$ [11 females, 7 female neutered, 61 males, 1 male neutered, 1 male neutering status unknown]), HD (Labrador Retrievers, $n = 32$ [9 females, 7 female neutered, 14 males, 1 male neutered, 1 male unknown neutering status]), CCLR (Labrador Retrievers $n = 51$ [10 females, 19 female neutered, 15 males, 7 male neutered], Golden Retrievers 45 [5 females, 26 female neutered, 10 males, 4 male neutered]), and a population of dogs undergoing vaccination (Labrador Retrievers $n = 344$ [89 females, 29 female neutered, 105 males, 28 male neutered, 93 sex and neuter status unknown], Golden Retrievers $n = 94$ [42 females, 11 female neutered, 34 males, 6 male neutered, 1 sex and neutered status unknown]). The control samples were not

Table 1. Candidate genes selected for evaluation in canine OA and their SNP positions

Name	CFA	U	P	E	I	D	Function	Justification for evaluation inclusion	References
Ankyrin repeat domain 10 (ANKRD10)	22			2	3		Structural component of muscle	Increased expression in canine ruptured CCL	(Clements et al. 2008)
Adenosine triphosphatase, Class VI, Type 11B (ATP11B)	34				6		Cell membrane transport	Increased expression in canine ruptured CCL	(Clements et al. 2008)
Interleukin 1 alpha (IL1 α)	17		3	2	2		Proinflammatory cytokine	Increased expression in canine OA synovium and ruptured CCL. SNPs associated with human OA	(Loughlin et al. 2002; Smith et al. 2004, 2005; Maccoux et al. 2007)
Interleukin 10 (IL10)	7	3	2	3	3	2	Antiinflammatory cytokine	Increased expression in canine OA synovium	(Maccoux et al. 2007)
Interleukin 12 (IL12B)	4	5	4	2			Proinflammatory cytokine	Increased expression in human OA synovium	(Sakkas et al. 1998)
Interleukin 2 (IL2)	19				1		T and B cell proliferation	Expressed in human OA synovium	(Kardel et al. 2003)
Interleukin 4 (IL4)	11	1	1		4	2	Antiinflammatory cytokine	Demonstrates role in sexual dimorphisms OA susceptibility in experimental animal model	(Mahr et al. 2003)
Interleukin 6 (IL6)	14	2	2	1		3	Proinflammatory cytokine	Expressed in canine OA synovium and ruptured CCL	(Maccoux et al. 2007)
Leptin receptor (LEPR)	5			3			Adipokine receptor	Increased agonist (leptin) expression in human OA cartilage	(Dumond et al. 2003)
Matrix metalloproteinase 13 (MMP13)	5	2	6				Collagenase (cartilage break down)	Increased expression in canine hip OA cartilage and ruptured CCL	(Clements et al. 2006b, 2008)
Matrix metalloproteinase 3 (MMP3)	5				1	1	Collagenase (cartilage break down)	Increased expression in canine stifle OA	(Hegemann et al. 2003)
Matrix metalloproteinase 9 (MMP9)	24	2			1	1	Gelatinase	Increased expression in canine hip OA cartilage and ruptured CCL	(Clements et al. 2006b, 2008)
Secreted protein, acidic, cysteine rich (osteonectin/SPARC)	4		4		3		Matrix-associated protein	Increased expression in canine ruptured CCL	(Clements et al. 2008)
Tissue inhibitor of metalloproteinase 1 (TIMP1)	X	1				1	Inhibition of metalloproteinase activity	Increased expression in canine hip OA cartilage	(Clements et al. 2006b)
Tissue inhibitor of metalloproteinase 2 (TIMP2)	9				1		Inhibition of metalloproteinase activity	Reduced expression in canine hip OA cartilage and ruptured CCL	(Clements et al. 2006b, 2008)
Tissue inhibitor of metalloproteinase 3 (TIMP3)	10				1	1	Inhibition of metalloproteinase activity	Increased expression in human OA cartilage	(Kevorkian et al. 2004)
Tissue inhibitor of metalloproteinase 4 (TIMP4)	20	2				2	Inhibition of metalloproteinase activity	Reduced expression in canine hip cartilage OA	(Clements et al. 2006b)
Tenascin C (TNC)	11		3	2	2	1	Extracellular matrix protein	Increased expression in ruptured CCL/OA cartilage	(Salter 1993; Clements et al. 2006b, 2008)
Tumor necrosis factor alpha (TNF α)	12	2	1	1	3	2	Proinflammatory cytokine	Expressed by canine OA synovium	(Fujita et al. 2005; Hegemann et al. 2005)
Zinc finger, SWIM-type containing 2 (ZSWIM2)	36			2	2		Metabolism	Increased expression in OA cartilage and ligament	(Clements et al. 2008)

CFA, canine chromosome; U, upstream; P, Promotor; E, Exon; I, Intron; D, Downstream and their justification for evaluation as candidate genes.

evaluated for disease status. An internal genotyping control was included on each plate.

SNP Identification

Selected regions of each candidate gene were amplified by polymerase chain reaction (PCR). The PCR product was

assessed for the presence of a polymorphic product using denaturing high-performance liquid chromatography (Spiegelman et al. 2000), and amplicons with melt curve analyses indicating a SNP were sequenced. SNPs identified were annotated to a genomic position by alignment of the sequence with the canine genome (www.Ensembl.org), and

the Ensembl gene annotations (exon numbers, size, and position) were used to define the SNP positions. SNPs were designated a genomic location on the basis of their position (pre-gene $\leq 10\,000$ -bp upstream of exon 1, promoter <1000 bp of the start of exon 1, post-gene $\leq 10\,000$ -bp downstream of the last exon). Forty-six SNPs were selected from a previous study (*IL1 α* , *IL2*, *IL4*, *IL6*, *IL10*, *IL12 β* , and *TNF α* ; Short 2006; Short et al. 2009), 24 SNPs were identified as described (*ATP11B*, *ANKRD10*, *LEPR*, *MMP13*, *SPARC*, *TNC*, *ZSWIM2*). A further 43 SNPs were selected from the published canine genome sequence (www.ncbi.nlm.nih.gov) and the Broad Institute canine SNP database (<http://www.broad.mit.edu/tools/data/genvar.html>).

Genotyping

Genotyping was performed using the Sequenom MASSarray platform (Sequenom, Hamburg, Germany) as previously described (Short et al. 2007). Briefly, primers and probes were designed using the Sequenom assay design software version 3 and synthesized by Metabion AG (Martinsried, Germany). Primers and probes were pooled as recommended by the manufacturers instructions (<http://www.sequenom.com>). Multiplex PCR reactions, product cleanup, and probe extension reaction were performed in 384-well plates with 20 ng of DNA per well, using iPLEX Gold reagents. Samples were diluted and desalted with 6 mg of resin before dispensation onto a SpectroCHIP (Sequenom) using the Sequenom nanodispenser, before genotype identification by matrix-assisted laser desorption/ionization–time of flight mass spectrometry.

Data Analysis

Genotype and phenotype data were imported into BCgene software (www.bcplatforms.com), which was used to calculate genotyping rates, minor allele frequencies (MAFs), and Hardy–Weinberg equilibrium (HWE) for each control population. SNPs or individuals were not analyzed further if the call rates were below 80% or if the control population was not in HWE. Analysis of genotype associations with disease was performed by logistical regression to correct for the confounding variables (sex and neuter status) using PLINK, a web-based genetic analysis program (Purcell et al. 2007), with additive, dominant, and recessive models. The strength of the significant associations was checked with a multiple permutation test, using 10 000 permutations. Haplotype frequencies were calculated for each gene, and haplotype associations with disease were performed using logistical regression to correct for the confounding variables using PLINK. The strength of the associations was again checked with a multiple permutation test, using 10 000 permutations.

Estimates of statistical power were performed using the PS Power and Sample Size program (Dupont and Plummer 1998). When SNPs with a MAF ranging from 5% to 50% are used this study was powered to detect risk alleles with Odds Ratios (OR) ranges of 1.64–2.43 (Labrador Retrievers,

ED) to 2.10–3.46 (Golden Retrievers, CCLR) and protective alleles with OR ranges of 0.61–0.13 (Labrador Retrievers, ED) to 0.48–undetectable (Golden Retrievers, CCLR) if the allele is protective, at 80% power ($P < 0.05$).

Results

A total of 113 SNPs were analyzed; in Labrador Retrievers, 44% ($n = 54$) of SNPs were informative (MAF $>1\%$), 14% ($n = 16$) of SNPs demonstrated low heterozygosity (MAF $<1\%$), 15% ($n = 17$) were monoallelic, and 27% ($n = 30$) were not in HWE; in Golden Retrievers, 61% ($n = 69$) were informative, 27% ($n = 31$) were monoallelic, and 12% ($n = 13$) were not in HWE. The average genotyping rate for the Labrador Retrievers was 94.5% (range 81.3–99.6%), and 96.6% for the Golden Retrievers samples (range 84.9–100%). The concordance of the internal genotyping control between plates was 100%.

Case-control comparison of genotypes identified 10 SNPs whose minor alleles were significantly associated with risk ($n = 5$) or protection ($n = 5$) of orthopedic disease independent of confounding using the additive model (Table 2). The case-control comparison of haplotype frequencies identified 8 haplotypes that were associated with the risk ($n = 5$) or protection ($n = 3$) of orthopedic disease independent of confounding using the additive model (Table 3). One haplotype of *IL12 β* (CCTAACGG) was associated with the risk of developing ED and HD in Labrador Retrievers. Multiple permutation tests revealed statistical significance to be lost for all SNPs and haplotypes evaluated.

Discussion

A common genomic risk for joint disease and OA was not identified between 2 different breeds for the same disease or within the same breed for different diseases. Thus, our hypothesis that the genomic basis for the development of canine joint disease would be identified was not supported, although many other plausible candidate genes were not evaluated. Successful candidate gene studies of association identifying positive associations between a canine phenotype and genotype have been reported for diseases, such as diabetes mellitus (Short 2006). However, the overall success of such studies in identifying a mutation associated with a trait is extremely low in dogs when compared with linkage analysis (Aguirre-Hernandez and Sargan 2005). Although the collection of data for mapping studies is challenging from a client-owned dog population, whole-genome association have the power to identify the genomic regions which are associated with complex traits in dogs and thus which harbor candidate genes suitable for further evaluation (Karlsson and Lindblad-Toh 2008).

The analysis of multiple SNPs or haplotypes for association with a phenotype requires appropriate statistical correction to minimize the Type-1 statistical error (Balding

Table 2. The significant susceptibility SNP associations

Disease (breed)	Gene	SNP identity	SNP position	Position (CFA: base pair)	Minor allele	MAF control	Major allele	OR (+95% CI)	P value	Corrected P value
CCLR (GR)	ANKRD10	ANK_I2	Intron 2-3	22:62038934	A	0.47	G	0.31 (0.13–0.75)	0.009	0.089
CCLR (GR)	ANKRD10	rs23039786	Intron 1-2	22:62043702	G	0.46	A	0.33 (0.14–0.77)	0.011	0.123
CCLR (GR)	<i>TNC</i>	TNC_P4	Promotor	11:72171896	T	0.25	C	2.65 (1.25–5.61)	0.011	0.146
CCLR (GR)	<i>TNC</i>	TNC_E2	Exon 2	11:72170870	G	0.43	A	1.89 (1.01–3.54)	0.046	0.588
CCLR (GR)	<i>TNC</i>	rs22191687	Promotor	11:72171378	G	0.43	T	1.89 (1.01–3.54)	0.046	0.521
CCLR (GR)	<i>IL10</i>	rs24430111	Post-gene	7:8886928	A	0.39	G	0.32 (0.1–0.95)	0.041	0.480
ED (LR)	<i>IL12β</i>	12B_02W232	Pre-gene	4:54385156	A	0.15	T	0.51 (0.27–0.95)	0.033	0.594
ED (LR)	<i>IL12β</i>	12B_02Y190	Pre-gene	4:54385114	T	0.14	C	0.50 (0.26–0.95)	0.034	0.601
HD (LR)	<i>IL4</i>	4_13S97	Intron 2-3	11:23983033	G	0.18	C	2.04 (1.15–3.61)	0.015	0.298
HD (LR)	<i>IL4</i>	4_8R458	Intron 3-4	11:23984915	A	0.19	G	2.02 (1.14–3.58)	0.016	0.319

SNPs separated by breed (GR, Golden Retriever; LR, Labrador Retriever), disease (CCLR, cruciate disease; ED; HD), gene, SNP position, minor allele, minor frequency in controls (%C), major allele, the OR for the MAFs in the disease population with 95% confidence intervals (95% CIs) and statistical significance (*P* value) when compared by logistical regression, and the corrected *P* value determined by multiple permutation testing.

2006). The allele frequency of a number SNPs and haplotypes were observed to be associated with different joint disease phenotypes before correction by multiple permutation testing. In most cases, the ORs for SNPs associated with diseases evaluated in this study were below the limits calculated for 80% statistical power, and these limits were further reduced by the stratification of populations on the basis of confounding variables.

A proportion of our control cases were likely to have developed HD, ED, or CCLR with time, which would artificially lower the differences and significance of the changes in the MAFs recorded. Thus, the true ORs for the MAFs would probably be greater if control populations were available which had been accurately phenotyped for all 3 diseases. The true prevalence of each of these diseases in the control populations in the United Kingdom are unknown, so estimations of the degree of misclassification in the control population could not be made, as robust

epidemiological studies of orthopedic diseases have not been performed in dogs to date. Age was not included in the statistical model, but sex and neuter status were in view of previous associations with these factors with the diseases evaluated.

Conversely, it should be noted that the phenotype quality of the disease cohorts is extremely high, as all cases were at the extreme end of the phenotype. First, each case required surgery for their underlying condition and second, each case was diagnosed by a veterinary orthopedic specialist. Difficulties in obtaining appropriate numbers of samples to accurately determine differences in allele and genotype frequencies for case-control study of canine disease with dog populations are a recognized issue (Short et al. 2007).

The majority of SNPs we evaluated were in the intronic ($n = 33$) or promotor ($n = 28$) regions, in contrast to the predominantly exonic SNPs evaluated in successful human candidate gene association studies of OA (Valdes et al. 2004;

Table 3. The significant susceptibility haplotype associations

Disease (breed)	Gene	Number of haplotypes	Haplotype	Haplotype frequency (control)	OR	P value	Corrected P value
CCLR (GR)	ANKRD10	4	AGGG	0.54	2.53	0.010	0.184
CCLR (GR)	ANKRD10	4	GAGG	0.24	0.42	0.031	0.533
CCLR (GR)	<i>TNC</i>	5	CGGTTA	0.08	2.86	0.032	0.547
ED (LR)	<i>IL12β</i>	4	CCTAACGG	0.16	1.64	0.042	0.686
ED (LR)	<i>IL12β</i>	4	ATCTCTAG	0.14	0.52	0.045	0.710
ED (LR)	<i>TIMP3</i>	3	AT	0.36	0.65	0.049	0.739
HD (LR)	<i>IL4</i>	3	CCAGAG	0.19	2.06	0.014	0.273
CCLR (LR)	<i>IL12β</i>	4	CCTAACGG	0.17	1.98	0.021	0.394

Haplotypes separated by breed (GR, Golden Retriever, LR, Labrador Retriever), disease (CCLR, cruciate disease; ED; HD), gene, haplotype frequency in the control population, the OR for the haplotype frequency in the disease population, statistical significance (*P* value) when compared by logistical regression, and the correct and the corrected *P* value determined by multiple permutation testing. The SNP identity (chromosome: base pair) used for haplotypes for each gene were as follows: ANKRD10 (rs23039786 [22:62043702], ANK_I2 [22:62038934], ANK_I4 [22:62021737], rs23105168 [22:62018474]), *TNC* (TNC_P4 [11:72171896], rs22191687 [11:72171378], TNC_E2 [11:72170870], TNC_I4 [11:72165326], TNC_I24 [11:72112527], TNC_E25 [11:72112377]), *IL12B* (12B_01M115 [4:54384525], 12B_01Y90 [4:54384501], 12B_02Y190 [4:54385114], 12B_02W232 [4:54385156], 12B_02Y407 [4:54385331], 12B_03Y82 [4:54385528], 12B_03R196 [4:54385642], 12B_03R462 [4:54385908]), *TIMP3* (rs22007793 [10:33774314], rs22007745 [10:33749146]), *IL4* (rs22146864 [11:23976639], 4_13S97 [11:23983033], 4_12M397 [11:23982900], 4_8R458 [11:23984915], 4_2M351 [11:23987559], rs22189535 [11:23988181]).

Valdes, Van-Oene, et al. 2006). Ideally, exonic SNPs would have been evaluated for all genes, but few or none were identified in the genes screened. Although open-access canine SNP databases are available, detail is presently lacking for individual genes and was not always able to detect exonic SNPs in each candidate gene evaluated using our methodology.

Conclusions

A common genetic risk for the development of canine joint disease and OA was not identified between different diseases within a single breed, or for the same disease in two different breeds in this study of association with polymorphisms in 20 candidate genes. Spurious association of candidate gene allele frequencies is readily identified in candidate genes if data are not appropriately corrected for multiple testing.

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References

- Aguirre-Hernandez J, Sargan DR. 2005. Evaluation of candidate genes in the absence of positional information: a poor bet on a blind dog! *J Hered*. 96:475–484.
- Balding DJ. 2006. A tutorial on statistical methods for population association studies. *Nat Rev Genet*. 7:781–791.
- Chase K, Lawler DF, Carrier DR, Lark KG. 2005. Genetic regulation of osteoarthritis: a QTL regulating cranial and caudal acetabular osteophyte formation in the hip joint of the dog (*Canis familiaris*). *Am J Med Genet*. 135:334–335.
- Clements DN, Carter SD, Innes JF, Ollier WE. 2006a. Genetic basis of secondary osteoarthritis in dogs with joint dysplasia. *Am J Vet Res*. 67:909–918.
- Clements DN, Carter SD, Innes JF, Ollier WE, Day PJ. 2006b. Analysis of normal and osteoarthritic canine cartilage mRNA expression by quantitative-PCR. *Arthritis Res Ther*. 8:R158.
- Clements DN, Carter SD, Innes JF, Ollier WER, Day PJ. 2008. Gene expression profiling of normal and ruptured canine anterior cruciate ligaments. *Osteoarthritis Cartilage*. 16:195–203.
- Dumond H, Presle N, Terlain B, Mainard D, Loeuille D, Netter P, Pottier P. 2003. Evidence for a key role of leptin in osteoarthritis. *Arthritis Rheum*. 48:3118–3129.
- Dupont WD, Plummer WD. 1998. Power and sample size calculations for studies involving linear regression. *Control Clin Trials*. 19:589–601.
- Duval JM, Budsberg SC, Flo GL, Sammarco JL. 1999. Breed, sex, and body weight as risk factors for rupture of the cranial cruciate ligament in young dogs. *J Am Vet Med Assoc*. 215:811–814.
- Fujita Y, Hara Y, Nezu Y, Yamaguchi S, Schulz KS, Tagawa M. 2005. Direct and indirect markers of cartilage metabolism in synovial fluid obtained from dogs with hip dysplasia and correlation with clinical and radiographic variables. *Am J Vet Res*. 66:2028–2033.
- Grondalen J, Lingaas F. 1991. Arthrosis in the elbow joint of young rapidly growing dogs—a genetic investigation. *J Small Anim Pract*. 32:460–464.
- Guthrie S. 1989. Some radiographic and clinical aspects of ununited anconeal process. *Vet Rec*. 124:661–662.
- Guthrie S, Pidduck HG. 1990. Heritability of elbow osteochondrosis within a closed population of dogs. *J Small Anim Pract*. 31:93–96.
- Hays L, Zhang Z, Mateescu RG, Lust G, Burton-Wurster NI, Todhunter RJ. 2007. Quantitative genetics of secondary hip joint osteoarthritis in a Labrador Retriever-Greyhound pedigree. *Am J Vet Res*. 68:35–41.
- Hegemann N, Wondimu A, Kohn B, Brunnberg L, Schmidt MF. 2005. Cytokine profile in canine immune-mediated polyarthritis and osteoarthritis. *Vet Comp Orth Trauma*. 18:67–72.
- Hegemann N, Wondimu A, Ullrich K, Schmidt MFG. 2003. Synovial MMP-3 and TIMP-1 levels and their correlation with cytokine expression in canine rheumatoid arthritis. *Vet Immunol Immunopathol*. 91:199–204.
- Janutta V, Hamann H, Klein S, Tellhelm B, Distl O. 2005. Genetic evaluation of elbow angles as predictors of elbow dysplasia in German shepherd dogs. *J Vet Med A Physiol Pathol Clin Med*. 52:254–261.
- Kardel R, Ulfgren A-K, Reinholt FP, Holmlund A. 2003. Inflammatory cell and cytokine patterns in patients with painful clicking and osteoarthritis in the temporomandibular joint. *Int J Oral Maxillofac Surg*. 32:390–396.
- Karlsson EK, Lindblad-Toh K. 2008. Leader of the pack: gene mapping in dogs and other model organisms. *Nat Rev Genet*. 9:713–725.
- Kevorkian L, Young DA, Darrah C, Donell ST, Shepstone L, Porter S, Brockbank SM, Edwards DR, Parker AE, Clark IM. 2004. Expression profiling of metalloproteinases and their inhibitors in cartilage. *Arthritis Rheum*. 50:131–141.
- LaFond E, Breur GJ, Austin CC. 2002. Breed susceptibility for developmental orthopedic diseases in dogs. *J Am Anim Hosp Assoc*. 38:467–477.
- Lang J, Busato A, Baumgartner D, Fluckiger M. 1995. Comparison of two classification protocols in the evaluation of elbow disease in the Bernese mountain dog. In: Abstracts of Papers Presented at the Annual Meeting of the International Elbow Working Group (IEWG). 1994 August 7; Philadelphia, PA. *Veterinary Radiology & Ultrasound*. 36. p. 170–171.
- Loughlin J, Dowling B, Mustafa Z, Chapman K. 2002. Association of the interleukin-1 gene cluster on chromosome 2q13 with knee osteoarthritis. *Arthritis Rheum*. 46:1519–1527.
- Maccoux LJ, Salway F, Day PJ, Clements DN. 2007. Expression profiling of select cytokines in canine osteoarthritis tissues. *Vet Immunol Immunopathol*. 118:59–67.
- Mahr S, Menard J, Krenn V, Muller B. 2003. Sexual dimorphism in the osteoarthritis of STR/ort mice may be linked to articular cytokines. *Ann Rheum Dis*. 62:1234–1237.
- Maki K, Liinamo AE, Ojala M. 2000. Estimates of genetic parameters for hip and elbow dysplasia in Finnish Rottweilers. *J Anim Sci*. 78: 1141–1148.
- Mateescu RG, Burton-Wurster NI, Tsai K, Phavaphutanon J, Zhang Z, Murphy KE, Lust G, Todhunter RJ. 2008. Identification of quantitative trait loci for osteoarthritis of hip joints in dogs. *Am J Vet Res*. 69:1294–1300.
- Nielen AL, Knol BW, van Hagen MA, van-der-Gaag I. 2003. Genetic and epidemiological investigation of a birth cohort of boxers. *Tijdschr Diergeneesk*. 128:586–590.
- Paradis M, Sauve F, Charest J, Refsal KR, Moreau M, Dupuis J. 2003. Effects of moderate to severe osteoarthritis on canine thyroid function. *Can Vet J*. 44:407–412.
- Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MAR, Bender D, Maller J, Sklar P, de Bakker PIW, Daly MJ, et al. 2007. PLINK: a tool set

- for whole-genome association and population-based linkage analyses. *Am J Hum Genet.* 81:559–575.
- Reed AL, Keller GG, Vogt DW, Ellersieck MR, Corley EA. 2000. Effect of dam and sire qualitative hip conformation scores on progeny hip conformation. *J Am Vet Med Assoc.* 217:675–680.
- Sakkas LI, Johanson NA, Scanzello CR, Platsoucas CD. 1998. Interleukin-12 is expressed by infiltrating macrophages and synovial lining cells in rheumatoid arthritis and osteoarthritis. *Cell Immunol.* 188:105–110.
- Salg KG, Temwiche J, Imholz S, Hazewinkel HA, Leegwater PA. 2006. Assessment of collagen genes involved in fragmented medial coronoid process development in Labrador Retrievers as determined by affected sibling-pair analysis. *Am J Vet Res.* 67:1713–1718.
- Salter DM. 1993. Tenascin is increased in cartilage and synovium from arthritic knees. *Br J Rheumatol.* 32:780–786.
- Short AD. 2006. The genetics of canine diabetes: a candidate gene study [PhD thesis]. [Manchester (UK)]: The University of Manchester.
- Short AD, Catchpole B, Kennedy LJ, Barnes A, Fretwell N, Jones C, Thomson W, Ollier WER. 2007. Analysis of candidate susceptibility genes in canine diabetes. *J Hered.* 98:518–525.
- Short AD, Catchpole B, Kennedy LJ, Barnes A, Lee AC, Jones CA, Fretwell N, Ollier WER. 2009. T cell cytokine gene polymorphisms in canine diabetes mellitus. *Vet Immunol Immunopathol.* 128:137–146.
- Smith AJ, Elson CJ, Perry MJ, Bidwell JL. 2005. Accuracy of haplotype association studies is enhanced by increasing number of polymorphic loci examined: comment on the article by Meulenbelt et al. *Arthritis Rheum.* 52:675–676.
- Smith AJ, Keen LJ, Billingham MJ, Perry MJ, Elson CJ, Kirwan JR, Sims JE, Doherty M, Spector TD, Bidwell JL. 2004. Extended haplotypes and linkage disequilibrium in the IL1R1-IL1A-IL1B-IL1RN gene cluster: association with knee osteoarthritis. *Genes Immun.* 5:451–460.
- Smith GK, Mayhew PD, Kapatkin AS, McKelvie PJ, Shofer FS, Gregor TP. 2001. Evaluation of risk factors for degenerative joint disease associated with hip dysplasia in German shepherd dogs, golden retrievers, Labrador retrievers, and Rottweilers. *J Am Vet Med Assoc.* 219:1719–1724.
- Spiegelman JL, Mindrinos MN, Oefner PJ. 2000. High-accuracy DNA sequence variation screening by DHPLC. *Biotechniques.* 29:1084–1090, 1092.
- Studdert VP, Lavelle RB, Beilharz RG, Mason TA. 1991. Clinical-features and heritability of osteochondrosis of the elbow in Labrador retrievers. *J Small Anim Pract.* 32:557–563.
- Todhunter RJ, Bliss SP, Casella G, Wu R, Lust G, Burton-Wurster NI, Williams AJ, Gilbert RO, Acland GM. 2003. Genetic structure of susceptibility traits for hip dysplasia and microsatellite informativeness of an outcrossed canine pedigree. *J Hered.* 94:39–48.
- Valdes AM, Hart DJ, Jones KA, Surdulescu G, Swarbrick P, Doyle DV, Schafer AJ, Spector TD. 2004. Association study of candidate genes for the prevalence and progression of knee osteoarthritis. *Arthritis Rheum.* 50:2497–2507.
- Valdes AM, Loughlin J, Oene MV, Chapman K, Surdulescu GL, Doherty M, Spector TD. 2007. Sex and ethnic differences in the association of ASPN, CALM1, COL2A1, COMP, and FRZB with genetic susceptibility to osteoarthritis of the knee. *Arthritis Rheum.* 56:137–146.
- Valdes AM, Doherty M, Spector TD. 2008. The additive effect of individual genes in predicting risk of knee osteoarthritis. *Ann Rheum Dis.* 67:124–127.
- Valdes AM, Van-Oene M, Hart DJ, Surdulescu GL, Loughlin J, Doherty M, Spector TD. 2006. Reproducible genetic associations between candidate genes and clinical knee osteoarthritis in men and women. *Arthritis Rheum.* 54:533–539.
- Whitehair JG, Vasseur PB, Willits NH. 1993. Epidemiology of cranial cruciate ligament rupture in dogs. *J Am Vet Med Assoc.* 203:1016–1019.
- Wilke VL, Conzemius MC, Rothschild MF. 2005. SNP detection and association analyses of candidate genes for rupture of the cranial cruciate ligament in the dog. *Anim Genet.* 36:519–521.
- Wilke VL, Conzemius MG, Kinghorn BP, Mcrossan PE, Weiguo C, Rothschild MF. 2006. Inheritance of rupture of the cranial cruciate ligament in Newfoundlands. *J Am Vet Med Assoc.* 228:61–64.
- Wood JLN, Lakhani KH, Dennis R. 2000. Heritability and epidemiology of canine hip-dysplasia score in flat-coated retrievers and Newfoundlands in the United Kingdom. *Prev Vet Med.* 46:75–86.
- Wood JLN, Lakhani KH, Rogers K. 2002. Heritability and epidemiology of canine hip-dysplasia score and its components in Labrador retrievers in the United Kingdom. *Prev Vet Med.* 55:95–108.

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